PATENT SPECIFICATION (11) 1 286 949

1286949

NO DRAWINGS

(21) Application No. 56340/70 (22) Filed 26 Nov. 1970

(31) Convention Application No. P 19 65 133.3

(32) Filed 27 Dec. 1969

(31) Convention Application No. P 20 49 938.1

(32) Filed 10 Oct. 1970 in

(33) Germany (DT)

(45) Complete Specification published 31 Aug. 1972

(51) International Classification A61K 27/00

(52) Index at acceptance A5B 350 35Y 380 38Y

(54) POLYMER-CONTAINING BILE ACID-FIXING AND TRIGLYCERIDE-FIXING COMPOSITIONS FOR ORAL ADMINISTRATION

(71) We, MERCK PATENT GESELLSCHAFT MIT BESCHRANKTER HAFTUNG, a German corporate body, of 61 Darmstadt, Frankfurter Strasse 250, Germany, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed to be particularly described in and by the following statement:—

The invention relates to novel pharmaceutical compositions which comprise nitrogen-containing polymers and that have, inter alia, bile acid-fixing and triglyceride-fixing activity.

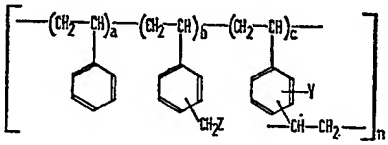
It is known, for example from British Specification No. 929,391, to use such polymers in oral pharmaceutical compositions for fixing bile acids in the digestive tract and lowering the cholesterol level in the blood. An example of such a composition is cholestyramine, a highly basic anion exchanger in chloride form, which is currently available for treating pruritus in conjunction with biliary engorgement.

We have found that certain synthetic, non-toxic, polymers containing ionisable amino groups fix bile acids more strongly in *in vitro* experiments than cholestyramine. They also have a triglyceride-fixing effect, which is not exhibited by cholestyramine. These polymers, which will be referred to hereinafter as "active substances", have, in contrast to cholestyramine, a macroporous structure and a swelling volume of less than 5 ml/g in water. After equilibration with air having a 100% relative atmospheric humidity at 25°C, they have a moisture content of less than 50%. This distinguishes them from the active substances described in British Specification No. 929,391, which have a moisture content higher than 65% under the same conditions.

The active substances also inhibit the lipase activity with respect to the fixed triglycerides and lower the cholesterol and triglyceride levels of the blood in *in vivo* experiments. The active substances are particularly valuable as lipase inhibitors if they are incorporated in diet mixtures for human beings or animals, to control weight gain. The compositions according to the invention may therefore be used for obesity and other diseases connected with excessive fat intake by the body.

The simultaneous presence of bile acid-fixing, triglyceride-fixing and lipase-inhibiting effects is regarded as a particular advantage of the active substances used according to the invention as compared with all known similar substances.

According to the present invention, therefore, we provide a composition having bile acid-fixing and triglyceride-fixing activity and suitable for oral administration, which comprises at least one synthetic, non-toxic, cross-linked polymer having the schematic structure:



in which Y is H or CH_2Z, Z is an ionisable amino group as herein defined, *a*, *b* and *c* indicate the relative weight proportions of the structural units derived from the respective monomers present in the polymer, *a* being from 1 to 20% by weight, *b* being from 60 to 94% by weight, and *c* being from 5 to 20% by weight, and *n* indicates the degree of polymerisation of the polymer said polymer also having a macroporous structure, a swelling volume of less than 5 ml/g in water, and a moisture content of less than 50% by weight, after equilibration with air having 100% relative humidity at 25°C, and at least one

solid, liquid or semi-liquid physiologically compatible carrier or excipient suitable for oral administration.

The term "ionisable amino group" is used in this specification to mean an amino group which forms a substituted ammonium salt on reaction with an acid and a quaternary ammonium salt on reaction with a quaternising agent. The active substances may accordingly be in the basic form (as primary, secondary or tertiary amines), in the form of ammonium hydroxides or in the form of acid-addition or quaternary ammonium salts.

The subscript *c* indicates the content of cross-linking agent, that is divinyl benzene or a derivative thereof. It is surprising that polymers of this chemical type with such a high content of cross-linking agent have the effects indicated, because British Specification No. 929,391, states that cross-linkage above about 5% seriously impairs the activity of these resins. The subscript *n* is a measure of the degree of polymerisation; owing to the three-dimensional cross-linkage precise figures cannot be given for *n*, but it is in any case greater than approximately 1000.

The group Z may, therefore, be an amino group, derived from a primary, secondary or tertiary amine of the partial formula R_2N— or an ammonium group derived from quaternary ammonium base of the partial formula $R_3N^+X^-$, in which the radicals R may be the same or different and are preferably H, or an alkyl or hydroxalkyl group containing, in particular, in each case 1 to 5 carbon atoms, particularly methyl, ethyl or 2-hydroxy-ethyl. Active substances in which two of the radicals R are methyl groups and the third radical R, which may be present, if desired, is a hydrogen atom or another methyl group, are particularly preferred.

The anion X^- is OH^- or an anion of any desired physiologically compatible acid. The active substances are usually administered in the form of chlorides. But the corresponding sulphates, phosphates, for example primary (dihydrogen), secondary (monohydrogen) or tertiary phosphates, bicarbonates, carbonates, formates, acetates, propionates, malonates, succinates, malates, tartrates, citrates, maleates, fumarates, ascorbates or polymers charged with the anions of saccharin or amino acids, such as aspartic acid or glutamic acid, may also be used.

Of the active substances in quaternary form, chlorides are particularly suitable. If these are administered in very high dosages, however, hyperchloraemic acidosis may occur in which the concentration of chloride ions in the serum increases, the concentration of bicarbonate ions in the serum decreases, there is an increased excretion of chloride ions in the urine and the pH value of the urine is reduced. These undesirable effects can be reduced to a minimum when the active substances have to be given in very high dosages, by using quaternary chlorides mixed with other quaternary salts, for example together with bicarbonates, phosphates or citrates.

The active substances can be prepared by conventional polymerisation, a relatively high content of cross-linking agent (5 to 20% and, preferably, 6 to 8% by weight) being used and the operation being carried out in the presence of inert substances which produce the macroporous structure of the polymers.

Of the basic anion exchangers available commercially, all those which have a macroporous structure are, in principle, suitable as active substances. For example, the products available under the following trade names can be used:
Amberlite IRA—68, Amberlite IRA—93, Amberlite IRA—401, Amberlite IRA 401—S, Amberlite IRA—900, Amberlite IRA—904, Amberlite IRA—910, Amberlite IRA—911, Amberlite XE—238, Amberlyst A—21, Amberlyst A—26, Amberlyst A—27, Amberlyst A—29, De-Acidite FFIP, De-Acidite HJP, De-Acidite KMP, De-Acidite MJP, De-Acidite NIP, De-Acidite PIP, Duolite A 101 D, Duolite A 101 D HI, Duolite A 102 D, Lewatit MP 60, Lewatit MP 62, Lewatit MP 64, Lewatit MP 500, Lewatit MP 600. ("Amberlite", "Amberlyst", Duolite" and "Lewatit" are Trade Marks).

These commercial products are preferably not used in the form in which they are obtainable commercially, but in a washed and, if desired, dried and/or ground, form so that many of the original characteristic values stated by the manufacturer, such as water content, bulk density, grain size and capacity, no longer apply.

Two commercial products have proved particularly suitable as active substances. They will be referred to hereinafter as "Active substance A" and "Active substance B". Their properties (characteristic data) in comparison with those of cholestyramine are shown in the following Table:

	Cholestyramine	Active Substance A	Active Substance B
Amine type	quaternary	tertiary	quaternary
Content of cross-linking agent (divinylbenzene)	2%	>5%	>5%
Capacity (dry)	2.9 meq/g	4.2 meq/g	3.6 meq/g
Swelling volume in water	21 ml/g	3.6 ml/g	3.25 ml/g
Bulk density (dry)	440 g/l	280 g/l	275 g/l
Water absorption at 25°C	>65%*	28%	36%

*According to British Specification No. 929,391.

These characteristic data were determined as follows:

Capacity

a) *Polymers of quaternary amine type*:

1 g of the dry polymer was converted into the OH form with 5% aqueous NaOH until chloride was no longer detectable in the filtrate. The polymer was washed with water until neutral, the OH anion was exchanged for the Cl anion by being washed in a column with 40 ml of 10% NaCl solution and then with 100 ml water. The base content of the filtrate was titrated with 0.1 N HCl, each 1 ml of HCl used corresponding to a capacity of 0.1 milli-equivalent (meq) per gram.

b) *Polymers of tertiary amine type*:

1 g of the dry polymer was stirred for 30 minutes with 50 ml of 1 n NaOH, then washed with water until free from alkali, and then mixed in a measuring flask with 10 ml of 1 N HCl. The flask was made up to 100 ml with water and left to stand for 16 hours with occasional shaking, and was then titrated with 0.1 N NaOH using methyl red.

Swelling volume:

1 g of the dry polymer was left to stand with a sufficient quantity (10—30 ml) of water for 2 hours with occasional shaking. The volume of the water-swollen polymer was then determined in a measuring cylinder after vibration for 1 minute.

Water absorption:

4 g of the polymer, dried to a constant weight at 60°C under reduced pressure, were exposed on a watch glass to a water-saturated atmosphere in a desiccator at 25°C until no more weight increase took place. The water absorption is indicated as a percentage of the total weight.

In comparison with cholestyramine, Active Substance A shows a 100% increase in lipoid fixing *in vitro*, while Active Substance B shows a 30% increase. ("Lipoids" means the sum of those substances that are fixed by the polymer according to the experimental method indicated below from a mixture of triglycerides and ox bile.) The fixing of bile acid by Active Substance A *in vitro* is approximately the same as that of cholestyramine; the fixing of bile acid by Active Substance B is about 50—60% higher than that of cholestyramine.

The following reductions of the cholestrol and triglyceride levels were obtained in *in vivo* experiments on lipid-fed rats (for method of analysis see Laffler, *American Journal of Clinical Pathology, 31,* 310 (1959) and Eggstein and Kreutz, *Klinische Wochenschrift, 44,* 262—267 (1966)).

	Cholesterol % reduction	Triglycerides % reduction
Cholestyramine	19	6
Active Substance A	22	21

The active substances also reduce the phospholipid content of the liver. For example, after treatment of rats with Active Substance A, the liver tissues of the animals showed a 70% reduction in the total lipid content in comparison with untreated controls and an 83% reduction of the phospholipid content (determined as phosphorus after incineration according to Quinland and Desega, *Analytical Chemistry, 27,* 1626 (1955)).

On the other hand, an 18% reduction of total lipids and a reduction of phospholipids of only 1% were observed after administration of cholestyramine under the same experimental conditions.

The active substances also favourably influence the lipid content of the liver. After homogenisation of deep-frozen liver specimens and extraction with chloroform/methanol (for method see Sperry, *Methods of Biochemical Analysis, 2,* 83 (1955)) the total cholestrol and triglyceride contents were determined by the above method. A reduction of the cholesterol content by 21% and of the triglyceride content by 36% was obtained with Active Substance A.

The active substances thus have two biological points of attack. They intervene in the enterohepatic circulation of bile acid and reduce its re-resorption. This process, just as with chlostyramine, leads secondarily to a lowering of the cholesterol level. In contrast to cholestyramine, however, they are also able to fix triglycerides to the same degree as cholesterol in the *in vivo* model and to inhibit resorption. This results in a lowering of the triglyceride level in the blood and is of great importance for the fat metabolism in the liver.

It has also been found that the activity of the active substance depends decisively on their particle size. The lipoid-fixing capacity increases as this decreases; the bile acid-fixing capacity, on the other hand, is not so dependent on the particle size. The ratio of lipoid to bile acid-fixing can therefore be adjusted to a desired degree by choice of the particle size of the active substance. This means that *in vivo* the ratio of the reduction of the cholesterol level to that of the triglyceride level can be varied or adjusted within certain limits according to the particle size of the active substance to be administered.

The particle size is generally less than 0.5 mm. In general, the active substances preferably have a particle size of from 0.0001 to 0.5 mm, and advantageously of from 0.001 to 0.2 mm. Active substances having particle sizes greater than 0.5 mm can be used and are effective, but as a rule are weaker in their effect than active substances of smaller particle sizes.

The influence of the particle size is shown, for example, by the following determinations using different degrees of fineness of Active Substance A:

	Particle size (mm)		
	0.2—0.36	0.125—0.2	0.024—0.125
Lipoid fixing (grams of lipoids per gram of active substance)	0.35	1.22	2.12
Bile acid-fixing (grams of bile acid per gram of active substance)	0.33	0.55	0.56
Lipase inhibition (%)	15%	61%	63%

These activities were determined as follows:

Lipoid fixing:

2 g of triglyceride mixture (olive oil) were emulsified in 80 ml of water with the addition of 1 g of ox bile (*Fal tauri*) with vigorous stirring. 1 g of active substance was added to the emulsion which was allowed to stand for 30 minutes with slight stirring; the pH was 6. The mixture was then filtered with suction, the solid residue was washed with water and dried at 60°C under reduced pressure until the weight was constant. The weight gain corresponds to the total quantity of lipoids fixed by the active substance.

Bile acid-fixing:

The procedure was the same as above, but without the presence of triglyceride. The increase in weight is regarded as a measure of the quantity of bile acids fixed by the active substance.

Lipase inhibition:

100 mg of olive oil were emulsified in 20 ml of an 0.1 M aqueous borate buffer of pH 8.5,

which also contained 0.3% albumin, 0.2% sodium desoxycholate and 0.2% $CaCl_2$. 100 mg of active substance were added. The reaction was started with 0.1 mg of lipase from pig pancreas and proceeded at 30°C with constant stirring. After 1, 2 and 4 hours, 5 ml of the mixture were titrated in each case to a pH of 11.0. A blind experiment containing no active substance was carried out in parallel. The percentage inhibition was calculated from the comparison of the results of the inhibition experiment and the blind test.

The lipase inhibition depends on the concentration ratio of active substance to triglyceride. If larger quantities of active substance are added, it may reach higher values. With 50, 200 and 400 (instead of 100) mg of Active Substance A (particle size 0.125—0.2mm), inhibitions of 48, 93 and 94% respectively were obtained by the method described.

The active substances are used in human or veterinary medicine in admixture with solid, liquid and/or semi-liquid excipients. Suitable excipients are organic or inorganic substances that are suitable for enteral administration and that do not react with the active substances, for example water, benzyl alcohol, polyethylene glycols, gelatine, lactose, starch, magnesium stearate or talcum. Suitable forms for enteral administration are tablets, dragees, pastilles, capsules, but more preferably granulates, powders, jellies, sweets, suspensions, syrups or juices. These compositions may be sterilised, if required, or adjuvants may be added to them such as lubricants, preservatives, stabilisers or wetting agents, emulsifiers, salts to influence the osmotic pressure, buffer substances, or colouring, flavouring and/or perfuming substances.

The active substances may, if desired, be given in combination with one or more other active materials. For example, the active substances may be ingredients of a low-fat diet or a slimming diet and may be administered together with nutrients, for example proteins, carbohydrates, vitamins, such as vitamins A, B_1, B_2, B_6, B_{12}, C, D_2, D_3, E and/or sweeteners, such as saccharin.

The active substances are preferably given in doses of 0.2 to 10 g per dosage unit; the daily dose is advantageously from 1 to 30 g. Owing to the low toxicity of the active substances, however, much higher doses, for example up to 200 g a day, may be given.

The active substances are substantially odourless and tastless and thus resemble cholestyramine. Owing to their very low swelling capacity, however, they lend themselves very well to galenic processing and in this respect are far superior to cholestyramine. The administration of the active substances only demands a small supply of extra liquid, whereas considerable quantities of liquid have to be supplied to the body when active materials, such as cholestyramine, with pronounced swelling properties are taken. This is particularly important, because all these substances have to be taken in relatively large doses.

In order that the invention may be more fully understood, the following examples are given by way of illustration only:—

Example A

Working up of a commercial product

The starting material was a macroporous, commercially available basic anion exchanger having generally spherical particles and possessing the following characteristics:

Particle size:	0.3 — 1.0 mm
Bulk density of swollen resin:	600 — 680 g/l
Specific loading:	up to 40 l/h l
Total capacity (Swollen resin):	1.8 mval/ml
Useful capacity:	up to 38, under special conditions up to 45 g (CaO/l resin)
Temperature stability:	up to 100°C

The material having a particle size of less than 0.5 mm was removed from the commercial product by wet sieving. 100 g of this finer fraction was washed in a column with 300 ml of methanol and then quantitatively converted into the chloride form with 2 N HCl. The material was washed with twice-distilled water until acid-free and dried for 48 hours under reduced pressure at 60°C. The resulting product was comminuted for 12 hours or longer in a mortar (Retsch mill). Dry sieving yielded fractions of 0.024 to 0.125 mm, 0.125 to 0.2 mm and 0.2 to 0.36 mm particle size of Active Substance A.

Pharmaceutical preparations according to the invention are described in the following examples by way of illustration:

Example 1:

Sweets

Each sweet had the following composition:

Active substance	5 g
Fruit pulp (dried):	3 g
Pectin:	0.5 g
Citric acid:	0.5 g
Ascorbic acid:	0.1 g
Preservative (e.g. sorbic acid):	0.1 g
Cane sugar:	10 g
Water:	10.8 g

Example 2:

Capsules

0.5 g of active substance was mixed with 5 mg of magnesium stearate. Capsules were filled with the mixture and coated with soft gelatin.

Example 3:

Dragees

Each dragee centre contained:

Active substance:	0.2 g
Lactose:	0.1 g
Wheat starch:	0.25 g
Talcum:	0.048 g
Calcium stearate:	0.002 g

The coating consisted of a mixture of wheat starch, sugar, talcum, finely divided silica and tragacanth.

Example 4:

Tablets

500 g of active substance and 100 g of potato starch were mixed, moistened with a solution of 2 ml of glycerin in 100 ml of 90% ethanol, granulated with 200 ml of aqueous 50% gelatine mucilage, the granulate was mixed with 50 g of talcum and 1 g of magnesium stearate and then pressed into 750 mg tablets. Each tablet contained 500 mg of active substance.

Example 5:

Syrup

A mixture was prepared from:

Active substance:	20 kg
Polyoxyethylene-sorbitan-fatty acid ester:	2 kg
Cane sugar:	35 g
Carboxymethyl cellulose:	0.5 kg
Preservative (e.g. mixture of p-hydroxy-benzoic acid methyl ester and p-hydroxy-benzoic acid-n-propyl ester):	0.1 kg
Ascorbic acid:	0.5 kg
Glycerin:	2 kg
Flavouring and colouring agents:	as required

and this was made up to 100 litres with distilled water. A dose unit (5 ml) contained 1 g of active substance.

Example 6:

Aqueous suspension

30 kg of glycerol were mixed with 1 kg of agar-agar, this mixture was added to 40 litres of water, the whole was stirred until the mixture was homogeneous, and it was then heated to 50°C. 20 kg of active substance and 150 g of saccharin sodium were added, together with colouring and flavouring agents as required, and the whole was made up to 100 litres with water. A dose unit (5 ml) contained 1 g of active substance.

Example 7:

Powder

A mixture was prepared from:

Active substance:	70 kg
Sodium alginate:	4 kg
Gum arabic:	26 kg

and this was pulverised until fine. The resulting powder was sealed in air-tight unit packs of 5 g each. Shortly before use, one or more packs were opened, the contents were suspended by stirring in a liquid or semi-liquid carrier, e.g. water, fruit juices, vegetable juices or non-alcoholic drinks, such as milk, or apple sauce and given in this form.

Example 8:

Granulate

3 g of flavouring substances were dissolved in 100 ml of ethanol, the solution was mixed with 797 g of glucose, and the resulting aromatized glucose was added to a mixture of 10 kg active substance, 300 g sodium alginate and 400 g of polyacrylic acid. The whole was mixed well, granulated and dried in the air. Air-tight polyetheylene bags, each containing 4 g of the granulate, were then filled with the substance.

Example 9:

Powder

5 g of active substance were pulverized until very fine, and unit packs of 5 g each were filled with them and sealed so as to be air-tight. Shortly before use, one or more packs were opened, the contents were suspended by stirring in a liquid or semi-liquid carrier, for example water, fruit juices, vegetable juices or non-alcoholic drinks, such as milk, or apple sauce, and given in this form.

Example 10:

Aqueous suspension

1 kg of active substance, 100 g of pectin and 10 g of sorbic acid were stirred with pure water so that the total volume of the suspension was 10 litres. A does unit (5 ml) contained 0.5 g of active substance.

The active substances must be given in relatively large quantities if they are to be used for triglyceride fixation. Assuming an average fat intake of 60 g a day and a fat-fixing capacity of the active substances equivalent to twice their weight, 30 g of active substance would have to be given, for example, to fix the total fat intake. The traditional pharmaceutical formulations are not very suitable for such large quantities of active substance. It is better, in this case, to give the active substances together with foodstuffs, for example in the form of foods or drinks to be taken before, during or after meals, such as cocktails, juices, pastries or dessert.

Example 11:

Fruit-juice drink

150 litres of fruit juice, e.g. orange, tomato or currant juice, were mixed with 1 kg of sodium alginate, 30 kg of active substance and 0.1 kg of sorbic acid. A dose unit (180 ml) contained about 30 g of active substance.

Example 12:

Pastry

50 g of margarine and 2 eggs (95 g) were stirred with 175 g of sugar. 250 g of oat flakes, 50 g of flour, 300 g of active substance, 8 g of commercial baking powder, flavouring substances as desired, and water were added gradually, enough of the latter (375 g) being added to form a kneadable dough. After baking, 895 g of pastry with an active-substance content of 33.5% were obtained. A cake weighing 15 g thus contained about 5 g of active substance.

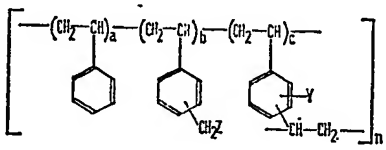
Example 13:

Pastry

The procedure in Example 12 was followed, but without the addition of margarine and with 200 g of sugar. 860 g of practically fat-free pastry with an active substance-content of 34.8% were obtained; a daily dose of 6 cakes of about 15 g each (before, during or after meals, corresponds to the administration of 30 g of active substance. Similar pastries may be prepared in which the sugar is replaced by sorbitol or physiologically compatible sweeteners, such as saccharin sodium.

WHAT WE CLAIM IS:—

1. A composition having bile acid-fixing and triglyceride-fixing activity and suitable for oral administration, which comprises at least one synthetic, non-toxic, cross-linked polymer having the schematic structure:



in which Y is H or CH_2Z, Z is an ionisable amino group as herein defined, *a*, *b* and *c* indicate the relative weight proportions of the structural units derived from the respective monomers present in the polymer, *a* being from 1 to 20% by weight, *b* being from 60 to 94% by weight, and *c* being from 5 to 20% by weight, and *n* indicates the degree of polymerisation of the polymer said polymer also having a macroporous structure, a swelling volume of less than 5 ml/g in water, and a moisture content of less than 50% by weight, after equilibration with air having 100% relative humidity at 25°C, and at least one solid, liquid or semi-liquid physiologically compatible carrier or excipient suitable for oral administration.

2. A composition according to claim 1 which is in dosage unit form, each dosage unit containing from 0.2 to 10 g of the polymer.

3. A composition according to claim 1 or 2, in which the polymer has a particle size of from 0.0001 to 0.5 mm.

4. A composition according to any of claims 1 to 3, in which Z is a dialkylamino group.

5. A composition according to claim 4, in which the value of *c* is from 6 to 8% by weight and the polymer has a swelling volume of 3.2 to 4 ml/g, a moisture content of from 20 to 40% by weight after equilibration with air having 100% relative humidity at 25°C, and a particle size of from 0.0001 to 0.2 mm.

6. A composition according to any of claims 1 to 5, which additionally comprises at least one other active material.

7. A composition having bile acid-fixing and triglyceride-fixing activity substantially as herein described in any of Examples 1 to 13.

A. A. THORNTON & CO.,
Chartered Patent Agents,
Northumberland House,
303/306 High Holborn,
London, W.C.1.

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1972.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.